RADIOECOLOGY

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TECHNIQUES FOR STUDYING MOVEMENTS OF VERTEBRATES IN THE FIELD

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INTRODUCTION

Labeling animals with radionuclides and tracing the movements of these animals with portable detectors is an effective method for studying certain aspects of animal behavior in a natural environment. This paper consists of a general description of the method and a review of its applications with vertebrates.

The first recorded use of radionuclides in tracing movements of animals is reported by Tomes and Brian (1946). They labeled Elaterid beetles of the genus Agriotes with radium sulfate sandwiched between aluminum discs which were cemented beneath the elytra. Labeled beetles could be located under four inches of soil with a Geiger-Mueller rate meter.

Griffin (1952) published the first account of the use of radionuclide labels to study vertebrate activity. He obtained data on nest-attentiveness of a labeled Semipalmated Plover (Charadrius semipalmatus).

Radionuclide labeling is particularly useful for studying animals which are secretive in their movements. For example, it is difficult to observe small mammals or amphibians living in areas with dense vegetational cover. Individuals marked by the conventional methods of toe-clipping or banding are difficult to relocate, whereas animals labeled with radionuclides are relatively easy to locate in this type of habitat. Similarly some animals that spend all or part of their lives underground may be studied by this method.

GENERAL DESCRIPTION OF METHOD

In general the technique consists of placing a small amount of radionuclide on an animal in such a way that the animal is not seriously injured by the radiation and behaves in a normal manner. After being labeled the animal is released in its home range and its movements are followed by periodically scanning the area with a portable detection device. In most cases workers have made periodic checks at 12-or 24-hour intervals of the animal's location. In a few instances labeled animals have been tracked continuously for short periods of time (Godfrey, 1955; Green and Spinks, 1955).

This method of labeling animals has both advantages and disadvantages. The two most significant advantages are that in many situations observations can be made without interfering with the natural behavior of the organism and that labeled animals can be located without actually being seen by the observer. Another advantage is that radioactive labels are frequently not detectable by the labeled organisms or by predators that might prey differentially upon marked animals.

Use of radioactive materials which are metabolically incorporated into the tissues of the organism provides several additional advantages. Animals may be labeled and studied without ever being seen or handled by the observer. Many individuals of a population can be marked with a minimal effort if the radionuclide is administered to the organisms through their food. In these cases the radioactive label becomes a part of the animal during the course of the experiment.

A further advantage of certain metabolically incorporated radioactive material is that the nuclide can be transferred to young across the placenta (Finkel, 1947) and to chicks through the egg (Driggers et al., 1951; McCabe and LePage, 1958; Spinks et al., 1949). This type of transfer may be useful in studying dispersal of young from the place of birth or for the identification of specific litters or broods.

Portions of certain metabolically incorporated radioactive labels are eliminated in feces and urine. These signs may then be used to indicate the presence of the labeled animal.

The use of radionuclides in field studies has numerous disadvantages. One of the most serious is that of potential radiation hazard. The scientist must consider the possibilities of danger to himself and other human beings as well as to the ecosystem in which he is working. The choice of a study area must take into account not only the range of the species being labeled but also the range of possible predators on that species.

Standards for safe handling of radioactive material are available from the National Bureau of Standards and papers on health physics can be obtained from the Division of Technical Information, U. S. Atomic Energy Commission. Anthony and Norris (1946) and Comar (1955) discuss some of the problems of safety while injecting animals with radionuclides.

The effects of radioactive labels on the individual animal are likely to be more significant to the investigator than the over-all effects on the ecosystem. One must use extreme care to minimize the chances of radiation injury affecting the behavior and movements of the labeled organism. Control studies with non-radionuclide labeled animals should be included in investigations whenever possible.

Griffin (1952) gives a detailed discussion of the calculation of the radiation dose to a labeled animal and its likely physiological effect. His evaluation is based in part on the assumption that the labeled animal will have approximately the same threshold for radiation injury as the human skin. He concluded that the maximum permissible dose for "wild birds" would be 1,000 roentgens.

In evaluating the effects of a radioactive label one must consider the label as producing whole-body radiation which may cause injuries at lower dose rates than when radiation is restricted to a portion of the organism (Claus, 1958).

Effects of a given amount of radiation would probably be lower on poikilotherms than on homiotherms due to the comparatively low body temperature of the former during extended periods of the year. This concept is supported by studies by Patt and Swift (1948) who found that survival time of frogs (Rana pipiens) exposed to x-radiation could be prolonged by maintaining them low temperatures.

A somewhat analogous situation might be expected to exist between survival of hibernating and non-hibernating mammals. The onset of radiation damage was delayed but not prevented when marmots (Marmota monax) (Brace, 1952) and ground squirrels (Spermophilus tridecemlineatus) (Doull and Du Bois, 1953) were irradiated while in hibernation.

Another disadvantage to this method of labeling vertebrates is that it is difficult to distinguish between labeled individuals when there are many in the same area. It is possible to identify a few animals positively when labels of different strengths are used. Variation between labels should be in the order of 100 microcuries.

Griffin (1952) and Pendleton (1956) discuss in detail these and other advantages and disadvantages of using radionuclides to trace animal movements.

The selection of the nuclide must be based on the nature of the experiment with special consideration being given to the organism and its environment. Choice of an inert or metabolic-incorporated label must be made and characteristics of available radionuclides such as type and energy of radiation, physical half-life, biological half-life (time in which animal eliminates one half of administered dose by regular processes), and toxicity must be known.

In most vertebrate tracking studies an inert nuclide emitting gamma rays at an energy greater than one million electron volts would be preferable. This type of radiation could be detected at a greater range and through more intervening soil, water, or vegetation than weak gamma or alpha or beta radiation. In most cases alpha and beta radiation increase the radiation dose to the organism without facilitating the tracking of the animal. For inert labels a nuclide with a physical half-life in the range of 100 days to several years would probably be most appropriate.

Pendleton's (1956) review and bibliography should be examined prior to selection of a nuclide.

When an inert label, such as a piece of metal wire or rod, is desired the values of encapsulating must be considered. Griffin (1952), Godfrey (1954), Karlstrom (1957), Linn and Shillito (1960), and others have encapsulated labels in lead, aluminum, nickel, and other materials to facilitate handling of the nuclide and to shield the organism from beta radiation. Breckenridge and Tester (1961), Kaye (1960, 1961), and others have successfully used wire labels without encapsulation. In studies where the radioactive label emits large amounts of beta radiation and where the label must remain on the animal for extended periods of time encapsulation may be desirable. My investigations with tags containing 100 microcuries tantalum-182 used over one year on three species of Bufo indicate that encapsulation is not necessary when genetic damage can be ignored.

Techniques of labeling individuals vary considerably but can be logically separated into methods of attaching inert labels and methods of incorporating metabolic labels. Leg rings with radioactive material attached have been used on birds and mammals by Godfrey (1954, 1955), Griffin (1952), and Linn and Shillito (1960). Nora Croine-Michielsen of the University of Leiden marked small mammals with silver leg rings made radioactive by neutron activation in a reactor (Linn and Shillito, 1960). Breckenridge and Tester (1961) and Kaye (1960) used hypodermic needles with stylets for inserting wire labels subcutaneously in toads and mammals while Karlstrom (1957) labeled amphibians by cutting the skin and inserting a label with a forceps.

Jenkins (1954) applied a metabolically incorporated label to a small mammal by feeding "spiked" food whereas Miller (1957) injected radioactive material in similar animals to obtain longer retention in the body. McCabe and LePage (1958) made intramuscular implantations of gelatinous capsules containing calcium-45 in birds. Rapid administration of labels to numerous individuals could be facilitated by the use of oral capsules or implantation guns available from medical supply houses.

Problems of detection of labeled animals are minimized by using the most sensitive portable instrument available to the study. Instruments with directional shields or with electronic filters capable of reducing the response to radiation with energies above and below that emitted by the nuclide would be desirable. These modifications would cut out some background radiation resulting in an improved signal-to-noise ratio. My own preliminary tests with one-inch lead directional shields attached to the probe of a scintillation counter have not shown any increase in range of detection on 100 microcurie tantalum-182 sources. It is possible that the amount of shielding required to bring about substantial increase in detection distance would be too heavy for a portable instrument.

Distances at which labels can be located vary with the amount of radionuclide, the energy of radiation of the nuclide, and the amount and atomic weight of substances between the label and detector. Griffin (1952) discusses the practicable range of detection and gives a formula for its determination. Karlstrom (1957) conducted experiments on the range of detection of various quantities (20 to 2,800 microcuries) of cobalt-60 buried at selected depths in loam topsoil.

Detection distances reported in the literature vary from a few inches for feces and urine containing phosphorus-32 (Miller, 1957) to 23 feet for a 2,800 microcurie label of cobalt-60 with only air intervening between the label and scintillation counter (Karlstrom, 1957). This level of radioactivity was considered too high to allow use of the label on an animal. Labels of 100 microcuries of tantalum-182 were located on the ground surface from 15 to 20 feet while a buried toad carrying a four-month-old label (100 microcurie source initially) was located 22 inches deep in silty clay loam by Breckenridge and Tester (1961). We were able to detect animals marked with relatively undecayed tantalum-182 in 16 inches of water from a distance of four to five feet.

Labeled animals can be located with scintillation or Geiger-Mueller counters by systematic coverage of a study area or by random searching. Karlstrom (1957) discusses these methods and their merits and applications. The development of an automatic system for tracking and recording the movements of a labeled vertebrate similar to the device designed by Green and Spinks (1955) for following soil-inhabiting wire worms would be a major contribution to movement-study techniques.

The following portion of this paper consists of a review of methods used to study vertebrate movements utilizing radionuclides presented by phylogenetic classes.

FISH

The marking of fish with radioactive labels presents serious safety problems and, consequently, the technique has been used in only a few instances. Several of these studies were conducted in the U.S.S.R.

Carp (Cyprinus carpio) and sturgeon (species not given) fry were labeled with phosphorus-32 by Shekhanova (1955). Carp fry 10 to 20 millimeters long were labeled by placing them in water (amount not stated) containing 220 microcuries of phosphorus-32 for two hours. Labeled individuals could be identified as long as 77 days after labeling. Sturgeon fry were found to pick up less phosphorus-32 than carp. Therefore, they were fed oligocheates which had been fed on a yeast moistened with a sodium salt solution containing phosphorus-32. The fish were fed for one to two days on labeled food and then considered labeled. A total of 72,500 sturgeon fry were successfully labeled by this method.

Karzinkin et al. (1959) used essentially the same technique to label 106,000 young sturgeon (Acipenser güldenstädti) to study migration.

A more simple technique for labeling is discussed by Bogoiavlenskaia (1959) who added calcium-45 directly to the surrounding water. He found that the physiological uptake of the nuclide resulted in an accumulation in the bony structures of young sturgeon (duration of exposure not stated in translation). The recommended dosage is 300 microcuries calcium-45 per liter of water when the concentration of stable calcium is between 43 and 72 milligrams per liter. This dosage served as a label with a useful life of approximately one year.

In North America radionuclides have been used to study the population dynamics of Alaska herring (Clupea harengus pallasi). Wilimovsky (1961) presents a detailed description of the methods of labeling and detection and of the safety features of the system. In brief, Alnico V magnetic rods 0.5 inches long and 0.125 inches in diameter containing cobalt were numbered, imprinted with a return address, and gold-flashed. Rods were then neutron irradiated to produce cobalt-60. Labels were then coated with transparent polyethylene and injected into the abdominal cavity of the fish by means of a specially designed injector. Labeled herring are recovered from the commercial catch by equipment installed at processing plants. The label consists of ten microcuries of cobalt-60 and the recovery equipment is designed to be 100 per cent effective in removing labeled fish from the catch.

Scott (1961) successfully labeled 10- to 20-centimeter trout (Salvelinus fontinalis) by injecting one milliliter of solution containing 1.0 microcurie of iron-59 into the body cavity of each. The iron chloride solution was diluted with highly acid acriflavine hydrochloride before injection to prevent infection in the fish. Labeled fish were detected by placing a needle scintillation probe over the heart-liver region and counting with a portable rate meter or by placing the specimens in a circular tank with a detector at the center. The first method is faster where only a yes or no answer is required but involves more handling of each individual than the tank method.

Other methods for labeling fish with radionuclides and a review of the regulations governing the use of radionuclides which would apply to fish-labeling are presented by Seymour (1957). He concludes that radionuclide methods are generally not practical for labeling fish. Hooper et al. (1961) state that the only situations in which radionuclide labeling might be used are those in which there is complete control over the fish harvest or where the labeled fish will not constitute a public hazard. They suggest that radioactive labels would be superior to other methods in labeling at the egg or fry stage and following survival provided the preceding conditions can be met.

AMPHIBIANS

Use of radioactive labels in amphibians was initiated by Karlstrom (1957) who marked Yosemite Toads (Bufo canorus) with 20 to 30 microcuries of cobalt-60 contained in lead capsules. He was unable to locate any toads in hibernation sites four months after labeling and concluded that high background radiation and the comparatively low strength of his labels were responsible.

In laboratory experiments on survival of four species of western Bufo he found that many of the labeled toads lived long beyond the predicted period. For example, an adult male California Toad (Bufo boreas halophilus) survived 19 weeks exposure to 800 microcuries of cobalt-60 and two California toads carrying 2,000 microcurie cobalt-60 labels survived six and 14 weeks, respectively. Karlstrom states that the cause of death of these animals is unknown and that radiation "possibly contributed to the relatively early death."

Breckenridge and Tester (1961) report a high degree of success in following movements of the Manitoba Toad (Bufo hemiophrys). Animals labeled with 100 microcuries of tantalum-182 were followed during the summer for periods ranging from two to 90 days. Hibernation sites of six individuals were located in fall and the movements of these toads were followed until emergence eight to nine months later. Growth rates, movement, and behavior of non-labeled control animals and tantalum-182 labeled toads were similar in all respects.

At present we are securing depth determinations of burrowing animals by inserting three-inch fiberglass tubes into six-foot vertical holes dug in the soil about 12 inches laterally from the toads. The tubes, closed at the bottom to exclude water, are installed at the time the hibernation sites are located. The probe of the scintillation counter is lowered into each tube and the depth of the labeled toad is considered to be the depth at which the maximum radiation intensity is detected.

In our studies of the Plains Toad (Bufo cognatus) and the American Toad (B. terrestris americanus) we have had difficulties in relocating labeled individuals after several months. Plains Toads travel long distances and apparently most of our labeled animals have moved beyond the area which we can search. American Toads in our study area move comparatively short distances but spend most of their above-ground lives in dense brush and forest. The practical difficulties of searching in this habitat have prevented us from relocating most of the labeled individuals.

These results emphasize that behavior patterns of the organism and physical features of its habitat must be considered in the design of radionuclide tracking studies.

REPTILES

The only study of reptile movements using radioactive labels that I know of is currently being conducted by William Nelson of the University of Minnesota (personal communication). He is using essentially the same techniques and equipment developed by Breckenridge and Tester (1961) to investigate behavior of the Black-banded or Prairie Skink (Eumeces septentrionalis septentrionalis). His 100 microcurie tantalum-182 labels, 0.020 millimeters in diameter and 5.0 millimeters long, are injected subcutaneously in the mid-dorsal area and the place of injection is covered with a drop of collodion.

The animals under observation occupy a sandy ridge approximately 600 feet long and 55 feet wide adjacent to a small lake. The radionuclide labeling technique had enabled Nelson to follow individual skinks through two breeding seasons and to locate numerous underground nests and hibernation sites. The yearly range of movement of individual animals varied from about 15 feet for some females to 350 feet for one male. He attributes his success in keeping the labeled animals under observation to this comparatively small home range and to the tendency of the skinks to nest and hibernate at shallow depths in the soil.

BIRDS

Griffin (1952) labeled birds by attaching aluminum capsules containing zinc-65 (quantity not reported) to conventional bird bands in a study of nest attentiveness. A recording Geiger-Mueller rate meter with the tube buried near the nest was used. The apparatus was used primarily with the Semipalmated Plover and provided a continuous record of the periods when the labeled bird was present at the nest.

A somewhat similar system was developed by the National Research Council of Canada for the Canadian Wildlife Service to study nest attentiveness of Mallards (Anas platyrhyncos platyrhyncos) (Alex Dzubin, personal communication). A strontium-90 source was placed on one side of a nest and the tube of a recording Geiger-Mueller rate meter was placed on the opposite side. When the hen was on the nest the beta radiation was shielded from the counting tube and the record showed a lower count rate than when the hen was away. Numerous difficulties such as amplifier breakdown and "stray" vegetation interfering with the count rate led to abandonment of the techinque.

James Bendell (personal communication), University of British Columbia, reported labeling Blue Grouse (Dendragapus obscurus) with 500 microcuries of rubidium-86 attached to a standard patagial wing band. He was unsuccessful in locating these birds on their nests and attributes this to the difficulty of following the line of flight of a female returning to her nest. The range of detection with a Geiger rate meter was five to ten feet.

An indirect method of labeling progeny of specific females with radionuclides has been developed by McCabe and LePage (1958). They implanted calcium phosphate pellets containing calcium-45 in the breast muscles of Ring-necked Pheasant (Phasianus colchicus) hens prior to nesting to measure the contribution of the progeny of the released hens to the hunting take in the fall in Wisconsin. The nuclide was passed to the eggs and when leg bones of harvested birds were examined those bones from progeny of the labeled hens could be detected.

Tests on young from labeled hens showed significant accumulation of calcium-45 in the leg bones but extremely little in the breast muscle. McCabe and LePage (1958) concluded that eating the birds would not be in any way harmful.

I am currently initiating an investigation using modifications of this technique to analyze parasitic egg-laying in Redheads (Aythya americana). Individual hens in an enclosed study pen will be labeled with a specific radionuclide or combination of nuclides at the start of the 1963 breeding season. The radionuclide will be metabolically incorporated by the hen and some will be transferred to her eggs. The source of each egg will be determined by the energy of gamma radiation using a gamma ray spectrometer with a pulse-height analyzer. This technique will provide data for evaluation of the role of different age classes in parasitism and for analysis of the laying and incubation behavior of individual females.

MAMMALS

More studies utilizing radionuclide labels have been conducted on mammals than with any other class of vertebrates. All of these investigations have dealt with "small" mammals and both metabolically incorporated and inert labels have been used.

Jenkins (1954) fed a female lemming (Lemus sp.) 250 microcuries of phosphorus-32 mixed with rolled oats (amount not stated) and found that during the one month of study the animal could be easily identified in the field at a distance of eight feet with a Geiger-Mueller rate meter. Droppings from this animal containing the label served to determine its range of movement. This method facilitated the collection and study of lemming ectoparasites since the host could be located at feeding sites, middens, and resting places. One month after being labeled the lemming gave birth to a normal offspring which was labeled with phosphorus-32.

Home ranges of Meadow Mice (Microtus pennsylvanicus) were studied by Miller (1957) who injected phosphorus-32 into the animals and traced movements by detecting the nuclide in excretions on metal dropping boards distributed in the field.

In preliminary tests she injected House Mice (Mus musculus) with 200 microcuries of phosphorus-32 to determine the length of time the droppings would be labeled. For two weeks following injection any group of five fresh droppings from the labeled mice could be identified as labeled with a Geiger-Mueller rate meter at a distance of five centimeters.

Field studies revealed that both urine and droppings from labeled Meadow Mice contained phosphorus-32. Cleaning of the dropping boards in the field was difficult and the ultimate solution was to remove the phosphorus-32 contaminated boards from the field and replace them with new boards.

The rate of movement of food through the digestive tract was apparently spasmodic since some feces were high in phosphorus-32 and others were low or lacking. Miller (1957) concluded that 200 microcuries of phosphorus-32 was inadequate because of detection difficulties and recommends levels of 400 microcuries for animals the size of Microtus.

Several successful studies of movements of small mammals using radionuclide labels and a portable Geiger-Mueller rate meter have been reported by Godfrey (1953, 1954, 1955). She located 85 nests of Field Voles (Microtus agrestis) after labeling a large number of pregnant females with 100 microcurie tags of cobalt-60 held in a plaster of Paris mixture attached to a metal leg band (Godfrey, 1953).

European Moles (Talpa europea) were labeled for periods as long as three months with 80 microcuries of cobalt-60 contained in a metal ring fitted around the base of the tail (Godfrey, 1955). A Geiger-Mueller tube was mounted at the end of an eight foot bamboo pole so that animals could be located from a distance of at least ten feet. Labeled individuals could be readily located even when one foot below ground. The small home range (approximately 1/4 acre maximum) and slow movement of this mole made detection of its exact position relatively easy.

Kaye (1960, 1961) used comparatively large quantities of labels, varying from 700 to 4,500 microcuries of gold-198 to mark Eastern Harvest Mice (Reithrodontomys humilis humilis). Four animals were labeled with ten-millimeter pieces of 20-gauge wire in a study of movements and nest sites. Detection distances with a Geiger rate meter varied from 9 feet with a 700 microcurie label to 20 feet with 4,500 microcuries. The latter label was considered too large because the radiation intensity exceeded the capacity of the rate meter and made precise location of the animal difficult. He found headphones useful with his detector since increases over background were audible before becoming visible on the meter.

Other studies reviewed here suggest that labels in quantities used by Kaye (1960, 1961) might cause severe radiation damage to the mice. He does not mention this possibility or the effect the radiation might have had on natural behavior.

Linn and Shillito (1960) described rings containing radioactivity for use on shrews. Pieces of cobalt rod sheathed in nickel were irradiated to produce 50 microcuries of cobalt-60 and soldered to ordinary leg bands. The nickel and solder protected the animal from beta radiation but did not prevent detection of the gamma radiation by which the shrews were traced.

Another method of labeling shrews reported by Linn and Shillito (1960) is that used by Miss N. Croine-Michielsen of the University of Leiden. She tagged Sorex araneus with silver rings containing 200 microcuries silver-110. No beta radiation shield was used.

Homing and migration of bats of several species have been investigated using bands containing a radionuclide. Punt and Van Nieuwenhoven (1957) attached 250 microcurie rods of antimony-124 two by one millimeter to aluminum leg bands. Movement of bats within a cave and between caves was determined by periodically surveying the caves with a Geiger-Mueller rate meter with the tube mounted on a five meter extendable rod. Animals could be located even when in crevices out of sight of the observer.

Gifford and Griffin (1960) labeled bats with five microcuries of iodine-131. A solution containing the nuclide was poured into a flange of metal attached to a leg band. After the liquid had evaporated glue was spread over the remaining material and the flange was crimped against the main body of the band. Labeled bats could be located at distances up to one meter.

SUMMARY

A brief histroy of the use of radionuclides in tracing movements of animals is given and suggestions for use of this technique are made. The method consists of placing a small amount of radionuclide on an animal, releasing it in its home range and following its movements by periodically scanning the area with a portable detection device. Advantages and disadvantages of this method are discussed.

Methods used to study movements of fish, amphibians, reptiles, birds, and mammals utilizing radionuclide labels are reviewed.

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